

Amendment to the Specification

At page 6, line 9 and line 16, please insert the text - - (SEQ ID NO:14) - - after the text - gly-xxx-ser-xxx-gly -.

At page 6, line 10 and line 17, please insert the text 10, - - (SEQ ID NO:15) - - after the text - glu-xxx-xxx-leu-val-asp-gly -.

At page 6, line 26, please insert the text - - (SEQ ID NO:14) - - after the text - G-X-S-X-G - and please insert the text - - (SEQ ID NO:15) - - after the text - E-X-X-L-V-D-G -.

At page 9, line 13 please insert the text - - (SEQ ID NO:14) - - after the text – Gly-Xxx₁-Ser-Xxx₂-Gly -.

At page 9, line 14, please insert the text - - (SEQ ID NO:15) - - after the text – Glu-Xxx₃-Xxx₄-Leu-Val-Asp-Gly -.

As these amendments are to paragraphs within the specification, clean copies of each paragraph are included on the following pages before the Remarks with markings to show changes made.

Amendments: Markings to Show Changes Made

Page 6 Amendments:

The present invention provides a method for identifying a lipid acyl hydrolase having insect inhibitory properties comprising isolating and purifying a protein having lipid acyl hydrolase activity; obtaining a three dimensional crystal structure of said protein; and identifying the amino acid sequence of said protein; wherein said amino acid sequence contains a serine active site motif gly-xxx-ser-xxx-gly (SEQ ID NO:14) and an aspartate active site motif glu-xxx-xxx-leu-val-asp-gly (SEQ ID NO:15). Modifications of these motifs should disrupt the hydrolase and the insect inhibitory properties of the protein.

Furthermore, the invention provides a method of inhibiting insect infestation of a plant or plant part comprising providing in the insect's plant diet an insect inhibitory effective amount of a lipid acyl hydrolase having insect inhibitory properties when ingested by said insect, wherein the amino acid sequence of said hydrolase comprises a serine active site motif gly-xxx-ser-xxx-gly (SEQ ID NO:14) and an aspartate active site motif glu-xxx-xxx-leu-val-asp-gly (SEQ ID NO:15). The serine active site motif can be shown to be required by treating the hydrolase with a substrate which binds specifically and irreversibly to the serine in the serine active site motif, such as diisopropyl fluorophosphate. The serine active site motif and/or the aspartate active site motif can be shown to be required by modifying the amino acid sequence within each motif to show loss of function of hydrolase and insect inhibition.

The invention further provides a method for protecting a plant or part thereof against insect infestation comprising providing an insect controlling amount of a plant lipid acyl hydrolase protein having a crystal structure containing a serine active site motif G-X-S-X-G (SEQ ID NO:14) and an aspartate active site motif E-X-X-L-V-D-G (SEQ ID NO:15), each motif being present in the active site cleft defined by the crystal structure and the serine and aspartate residues in each motif being required for the catalytic function of the hydrolase, and the catalytic function of the hydrolase being required for functional and effective insect inhibition when provided in diet form to a susceptible insect larvae.

Page 9 Amendments:

An additional aspect of the present invention comprises applying an insect inhibitory effective amount of a protein sequence displaying lipid acyl hydrolase activity to a plant or incorporating said amount into said plant, wherein said protein sequence displaying lipid acyl hydrolase activity comprises a first peptide sequence comprising Gly-Xxx₁-Ser-Xxx₂-Gly (SEQ ID NO:14), and a second peptide sequence comprising Glu-Xxx₃-Xxx₄-Leu-Val-Asp-Gly (SEQ ID NO:15). Xxx₁ or Xxx₂ can be threonine or any other amino acid which is structurally and functionally similar to threonine. Xxx₃ can be an

aromatic amino acid residue, or preferably tyrosine or phenylalanine. Xxx₄ can be an amino acid residue considered in the art to be a base, preferably asparagine or histidine. A catalytic active site structure utilizing a serine-aspartate dyad chemistry is supported by the requirement for both peptide sequences being present, along with three dimensional modeling based on crystal structure of the protein sequence, and a pH rate profile indicating that a single residue with a pKa of less than about 5 must be deprotonated to show hydrolase activity and insect inhibitory bioactivity.